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Capillary Electrophoresis in Detection of Chemicals Related to the Chemical Weapons Convention

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1 **Introduction** 1
2 **Capillary Electrophoresis** 2
2.1 Capillary Zone Electrophoresis 2
2.2 Electrophoretic Mobility 2
2.3 Capillary Electrophoresis Separation, Efficiency, and Resolution 3
2.4 Limits of Detection 3
3 **Chemical Weapons Convention and Related Compounds** 3
3.1 Scheduled Chemicals 3
3.2 Degradation Products of Chemical Warfare Agents 4
4 **Capillary Electrophoretic Analysis of Chemical Weapons Convention Related Compounds** 4
4.1 Chromatographic Analysis of Chemical Weapons Convention Related Compounds 4
4.2 Capillary Electrophoresis Analysis of Organophosphates 5
4.3 Sulfur-containing Compounds 7
4.4 Toxins 8
5 **Applications** 9
5.1 Environmental Analysis 9
5.2 Quality Assurance 9
6 **Conclusion** 10
Acknowledgments 10
Abbreviations and Acronyms 11
Related Articles 11
References 11

This article describes the application of capillary electrophoresis (CE) to the analysis of compounds relevant to the Chemical Weapons Convention (CWC). CE is an analytical technique that employs narrow-bore, fused-silica capillaries to perform high-efficiency separations of analytes based on their mobilities in an electric field. In CE, analytes are dissolved in a buffer solution and placed in a capillary to which an electric field is applied. The analytes then migrate at a rate determined by their charge and size and are detected as they migrate past a detector. CE can analyze a broad range of compounds and is particularly applicable to the analysis of water-soluble degradation products of scheduled compounds under the CWC. Compounds typically analyzed by CE include the hydrolysis products of nerve agents such as the alkylphosphonates and hydrolytic or oxidative products of the sulfur mustards such as thiodiglycol. The alkyphosphonates require the use of indirect ultraviolet (UV) detection or other detection methods as they do not possess a suitable UV chromophore. Degradation products of the sulfur mustards have been analyzed by micellar electrokinetic chromatography (MEKC) and direct UV detection. The ability of CE to analyze anionic, cationic, zwitterionic, and neutral CWC-related compounds, whether they be chemical warfare (CW) degradation products or scheduled starting materials, without the requirement for elaborate sample processing or labeling procedures, is an important demonstration of the utility of CE for the analysis of CWC-related compounds. CE can be an important screening method for rapid sample processing and further analysis for unambiguous identification. The relative ease of analysis should also increase its utility in field and on-site analysis where minimizing the logistic burden is attractive.

1 INTRODUCTION

CE is an analytical technique that employs narrow-bore, fused-silica capillaries to perform high-efficiency separations of analytes based on their mobilities in an electric field.[1] The separation efficiency, which may be greater than 1 000 000 theoretical plates, is superior to that of many other chromatographic methods and is an important reason for the rapid growth of this technique.[2–4] In CE, analytes are dissolved in a buffer solution and placed in a capillary to which an electric field is applied. The analytes then migrate at a rate determined by their charge and size and are detected as they migrate past a detector. CE can analyze a broad range of compounds, has a simple instrument design which can be fully automated, and has very low volume requirements (nanoliter injection volumes are typical).[5]

The *Convention on the Prohibition of the Development, Production, Stockpiling, and Use of Chemical Weapons and their Destruction*, otherwise known as the CWC,[6] is an international treaty which will potentially eliminate the worldwide threat of CW.[7] CW agent degradation

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products or impurities can provide an indication of CW agent use, act as tracers to follow synthetic routes, or identify sources of precursors. For instance, analysis of samples taken during an allegation of use investigation were found to contain mustard (H) as well as other longer chain sulfur vesicants.[8] The identification of isopropyl methylphosphonic acid and methylphosphonic acid, decomposition products of the nerve agent isopropyl methylphosphonofluoridate (sarin (GB)), has also been reported in soil samples taken from bomb craters in Iraq years after the alleged attack.[9] More recently, isopropyl methylphosphonate has been detected in the urine[10] and serum[11] of persons exposed to GB during a terrorist attack in Japan.[12]

The degradation products of CW agents are often polar, water-soluble compounds. Thus analytical techniques which can directly analyze the target compounds without the need for derivatization are valuable for both on-site screening of samples and laboratory-based identification. CE is an important analytical method for the detection of these analytes because of the efficiency of separation, speed of analysis, and ease of operation. Degradation products of CW nerve agents are primary targets for analysis. However, these organophosphates are non-UV-absorbing and so are generally analyzed by indirect detection methods. Other compounds, such as the decomposition products of sulfur mustards, can be analyzed by direct UV detection but are non-ionizable and require the use of special CE experimental conditions. However, both classes of compounds have been readily analyzed, demonstrating the versatility of CE for the analysis of CWC-related compounds. The development of instruments for atmospheric pressure ionization mass spectrometry (MS) and the advent of commercial instruments for CE combined with electrospray ionization mass spectrometry (ESIMS) will greatly increase the importance of CE for the identification of CW agents and their degradation products.

2 CAPILLARY ELECTROPHORESIS

CE has been the subject of extensive reviews[13–16] and there are several books and publications describing the technique.[17,18] Key aspects of CE are discussed here to introduce CE and to review important fundamental issues for the analysis of CWC-related compounds. These issues can affect the quality of CE separations, detection of target compounds, and method development. Specialized CE methods, such as capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), and capillary electrochromatography (CEC) are beyond the scope of this article.

2.1 Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) has been used for the analysis of amino acids, peptides, ions, enantiomers, and many other ionic species. CZE is the simplest form of CE, employing a narrow-bore, bare fused-silica capillary (10–200 μm i.d., 370 μm o.d., polyimide coated) filled only with buffer. CZE achieves high-efficiency separations of both large and small molecules based on their mobilities in an electric field. The instrument design is fairly simple, consisting of two buffer reservoirs connected via a fused-silica capillary, a high-voltage power supply, and a detection system.[19]

Surface silanol groups of the bare fused-silica capillaries are partly ionized (SiO^-) in the presence of the electrophoretic medium, a run buffer in the pH range 2.5–10. This leads to the formation of a double layer of immobile cations tightly bound to the negative charges on the silica and a potential difference (zeta potential). Adjacent to this layer is a layer of weakly bound cations and their sphere of hydration. When a potential is applied across the capillary, these solvated cations migrate towards the cathode, resulting in a net flow of liquid through the capillary known as electroosmotic flow (EOF). The EOF is greater in magnitude than the mobility of the ions. Therefore, all analytes are swept in the same direction, regardless of charge. The zeta potential is also dependent on the ionic strength of the buffer. Increasing ionic strength decreases the zeta potential and thus the EOF. Selectivity in CZE is affected by the buffer pH and buffer additives (e.g. surfactants, EOF modifiers, or chiral selectors).

Since the EOF can be significantly greater than the electrophoretic mobilities of the individual ions in the sample, both anions and cations can be separated in the same run. Cations with the smallest mass-to-charge ratio are the fastest migrating species followed by cations with higher mass to charge ratios, then neutral species, and lastly anions. Anions migrate because they are swept to the cathode by the EOF. Control of the EOF is thus an important experimental consideration in method development and reproducibility of the analysis.[20–22] The conditions used during a CE separation can greatly affect the selectivity of the separation and so the run buffer selection and composition are extremely important to the success of any CE separation.

2.2 Electrophoretic Mobility

Identification of an analyte can be made on the basis of migration time or relative migration times, or by measurement of an analyte's electrophoretic mobility. Measurement of the electrophoretic mobility can compensate for variability in the migration time due to unstable electric

field, changes in buffer capacity or composition, temperature fluctuations, and changes to the capillary surface conditions.[23–25] The apparent electrophoretic mobility (μ_{app}) is characteristic for a given species at a certain pH. The apparent mobility results from the sum of the true electrophoretic mobility of the ion due to the electric field (μ_e) and the mobility due to the EOF (μ_{EOF}).

Apparent mobility (μ_{app}) can be calculated from the experimental data using Equation (1):

$$\mu_{app} = \frac{v}{E} = \frac{L_d}{t}\frac{L_t}{V} \quad (1)$$

where v is the velocity of an ion, E is the applied field strength ($V\,cm^{-1}$), L_d is the length from capillary to detector, L_t is the total length of capillary, t is the migration time, and V is the applied voltage.

In order to determine the electrophoretic mobility due solely to the attraction to the cathode (μ_e), a neutral EOF marker must be used to determine the mobility due to the EOF (μ_{EOF}), Equation (2):

$$\mu_e = \mu_{app} - \mu_{EOF} \quad (2)$$

Acetone, methanol, benzyl alcohol, and mesityl oxide can be used as EOF markers.

2.3 Capillary Electrophoresis Separation, Efficiency, and Resolution

Separations performed by CE are directly dependent on differences in the analyte mobilities and inversely dependent on the band broadening of the analytes as they migrate through the column. An important advantage of CE is that owing to EOF, the flow profile has a plug-shape as opposed to the parabolic flow profile of high-performance liquid chromatography (HPLC). This plug-shaped flow profile increases the resolution because the EOF is uniform throughout the capillary, resulting in much less band broadening due to frictional forces along the walls and pressure gradients across the column as is seen with pressure-driven systems.

Any factors contributing to band broadening will decrease the efficiency and resolution of the separation. A major source of band broadening in CE separations is the heat generated by the passage of electrical current through the fused-silica capillary. This is called Joule heat. The increase in temperature within the CE column depends on the power generated (power = voltage × current) and is determined by the capillary dimensions, conductivity of the buffer, and applied voltage. Excessive heat causes a nonuniform temperature gradient which changes the buffer viscosity across the capillary diameter. This results in different ion mobilities across the column, leading to band broadening. Thus an inherent advantage of performing CE in narrow-bore capillaries as compared with traditional electrophoresis is the rapid heat dissipation and reduction in the effects of heating. The efficiency expressed in theoretical plates (N) is given by Equation (3):

$$N = \mu_e \frac{EL_t}{D^2} \quad (3)$$

where μ_e is electrophoretic mobility, E is electric field, L_t is total length of the capillary, and D is the diffusion coefficient.[26]

It is evident from Equation (3) that the efficiency can be increased by increasing the electric field. However, this also results in increased Joule heating. Therefore, a balance between these two parameters must be achieved. Experimental parameters which can be optimized to limit Joule heating include decreasing the electric field (reducing the applied voltage), reducing the capillary internal diameter, decreasing the buffer ionic strength or concentration, and active temperature control.[27]

2.4 Limits of Detection

The limits of detection (LODs) of CE are comparable to those of other chromatographic methods. CE, because of its very low volume requirements, has very good mass sensitivity. Typical injection volumes are in the range 1–50 nL. Thus the often reported impressive picomole to femtomole mass detection limits are actually only in the low milligrams to high micrograms per liter (parts per million to parts per billion) range. Such concentrations are easily achieved by HPLC. The concentration limit of detection (CLOD), typically in the micromolar range, is higher than for HPLC where typical injection volumes of 1–10 μL allow for higher column loading.

Approaches to improvements in sensitivity include both precolumn and on-column sample concentration, derivatization with laser-induced fluorescence (LIF), and modification to the detection system.[28] On-column methods offer the advantage for trace analysis of minimizing the sample handling and possible loss of analyte. Three approaches are commonly used for sample concentration: sample stacking, sample focusing, and isotachophoresis. More recently, the development of on-line sample extraction devices such as solid-phase preconcentration phase CE has permitted injection volumes as high as 100 μL.[29]

3 CHEMICAL WEAPONS CONVENTION AND RELATED COMPOUNDS

3.1 Scheduled Chemicals

The CWC contains schedules of chemicals with specific verification and reporting requirements. The "Annex on

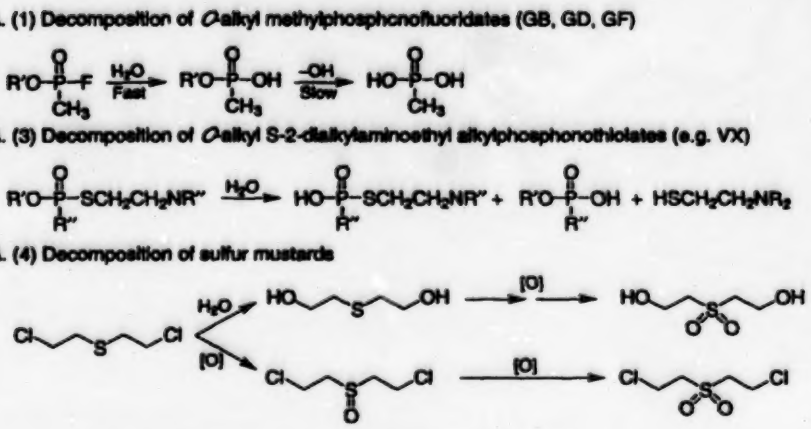


Figure 1 Major degradation pathways of Schedule 1 CW agents. GD, soman; GF, cyclohexyl sarin.

Chemicals" describes the guidelines for inclusion of a chemical on each of the three schedules. The Annex also lists specific classes, toxic chemicals, and their precursors for which specific reporting and verification measures are applied under the Verification Annex of the CWC. Each schedule is divided into toxic chemicals and precursors. Schedule 1.A lists those chemicals which pose a high risk to the CWC and which have been developed, produced, stockpiled, or used as chemical weapons. In addition to the toxic chemicals, certain chemicals and classes of chemicals are also scheduled because they can be used as immediate precursors for the production of Schedule 1 chemicals. These chemicals are seen to have little or no use for purposes not prohibited by the CWC.

The principal classes and chemicals under Schedule 1 of the CWC include classical CW agents such as the *O*-alkyl alkylphosphonofluoridate nerve agents (e.g. GB, CAS 107-44-8), the *N,N*-dialkylphosphoramidocyanidates (e.g. tabun (GA), CAS 77-81-6), and the *S*-dialkylaminoethyl alkylphosphonothiolates (e.g. *O*-ethyl *S*-2-diisopropylaminoethyl-methyl phosphonothiolate (VX), CAS 50782-69-9). Three vesicant (blister agent) classes are scheduled: the sulfur mustards (e.g. mustard gas (HD), CAS 505-60-2), the lewisites (e.g. Lewisite 1, CAS 541-25-3); and the nitrogen mustards (e.g. ethyl nitrogen mustard (HN-1), CAS 538-07-8). Two special compounds are also included in the schedule for which CE analysis has been applied in particular, the paralytic shellfish toxin saxitoxin (STX, CAS 35523-89-8) and 1.A.(8) the glycoprotein ricin (CAS 9009-86-3).

3.2 Degradation Products of Chemical Warfare Agents

The degradation and decontamination chemistry of CW agents has been reviewed previously.[30,31] Degradation can occur from natural weathering, as a result of decontamination, or by deliberate attempts to destroy or neutralize CW agents as might occur in a clandestine production facility. Figure 1 shows the major degradation pathways of Schedule 1 chemicals which lead to water-soluble products which can be analyzed by CE. *O*-Alkyl alkylphosphonofluoridate nerve agents such as GB undergo a two-step degradation in water or under alkaline conditions. The initial degradation product is isopropyl methylphosphonate[32] followed by a slower hydrolysis to methylphosphonic acid. Hydrolysis of *S*-dialkylaminoethyl alkylphosphonothiolates such as VX leads to the formation of *S*-2-diisopropylaminoethyl methylphosphonothiolate and ethyl methylphosphonate. Sulfur mustards can undergo both hydrolytic and oxidative decomposition. The primary hydrolysis product is thiodiglycol. Thiodiglycol sulfoxide and sulfone can arise directly from the hydrolysis of the oxidation products of mustard or the oxidation of thiodiglycol.

4 CAPILLARY ELECTROPHORETIC ANALYSIS OF CHEMICAL WEAPONS CONVENTION RELATED COMPOUNDS

4.1 Chromatographic Analysis of Chemical Weapons Convention Related Compounds

Unfortunately, as organophosphorus nerve agents readily undergo hydrolysis, identification of the intact CW agent is not always possible and the detection or identification of degradation products including alkyl methylphosphonic acids and methylphosphonic acid is more likely. Chromatographic methods, including ion chromatography (IC),[33,34] have been developed

for the tentative identification of these hydrolysis products. However, unambiguous identification requires the acquisition of spectrometric data from two different spectrometric techniques.[35,36] Proton and phosphorus nuclear magnetic resonance (NMR) spectroscopy have been applied to the trace analysis of CW agents and related compounds in rubber, paint, and soil.[37] MS and NMR has been used for the unambiguous identification of novel CW decomposition products relevant to verification.[38]

Hyphenated MS techniques such as with gas chromatography (GC), HPLC, or CE are preferred for multicomponent environmental samples. Nerve agent hydrolysis products, including a number of alkyl methylphosphonic acids and methylphosphonic acid, have been analyzed by gas chromatography/mass spectrometry (GC/MS) (see below). The polarity of these products required derivatization of the hydroxyl groups prior to GC separation and a number of derivatizing agents have been employed to enhance volatility. GC/MS methods have been reported for alkyl methylphosphonic acids, methylphosphonic acid, and related degradation compounds following *tert*-butyldimethylsilylation,[39] methylation with trimethylphenylammonium hydroxide,[40] trimethylsilylation,[34] and derivatization with pentafluorobenzyl bromide.[41] Derivatization methods allow the analyst to use the same GC/MS instrumentation as used for the CW agent analysis but suffer from the need to modify the intact scheduled compound prior to identification.

HPLC coupled with MS offers the opportunity to analyze the polar, ionic organophosphorus nerve agent hydrolysis products without the need for derivatization. Thermospray MS and tandem mass spectrometry (MS/MS) have been demonstrated for the identification of organophosphorus nerve agents and their hydrolysis products.[42–44] Since the mid-1990s, this ionization technique has been largely superseded by atmospheric pressure ionization methods, including electrospray (ES) and ionspray (IS), both of which have been applied to the analysis of organophosphorus nerve agent hydrolysis products.[45,46] CE offers an important alternative chromatographic technique for the separation of target compounds prior to MS analysis.

4.2 Capillary Electrophoresis Analysis of Organophosphates

The primary degradation products of the alkylphosphonofluoridate CW agents [Schedule 1.A.(1)],[1] the alkyl alkyphosphonates, do not have a UV chromophore or fluorophore suitable for direct detection. The most common detection mode for these compounds is indirect UV detection using a high UV-absorbing background electrolyte (BGE). Analysis of low-molecular-weight organic ions, such as methylphosphonic acid, has also been described as capillary ion electrophoresis (CIE). In these CIE methods EOF modifiers are used to reverse the EOF. Under these conditions, the anionic organophosphates comigrate with the EOF, a condition referred to as co-EOF. The EOF is modified using run buffer additives or by modifying the capillary surface.[47] Table 1 lists the organophosphate compounds analyzed by CE, including all modes of detection.

4.2.1 Indirect Ultraviolet Detection

In indirect detection, the analyte displaces a UV-absorbing BGE in the separation buffer. Detection is due to a decrease in the UV signal as the analyte, which has displaced the BGE, passes through the detector window.[48]

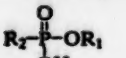
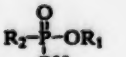
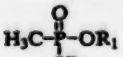
Detection is in effect done in a high-noise system owing to the strongly absorbing BGE. Thus detection limits are generally poorer than for direct UV methods. As shown in Equation (4):

$$\mathrm{CLOD} = \frac{C_{\mathrm{BGE}}}{RDr} \tag{4}$$

the CLOD is directly related to the concentration of the BGE (C_{BGE}) and inversely proportional to the displacement ratio (R) of the analyte and BGE and the dynamic reserve (Dr). The dynamic reserve is the ability of the detector to detect a small change in signal on top of a large signal.[49] Optimization of detection limits involves careful selection of BGE and the lowest C_{BGE} should be employed. The detector must provide a large dynamic reserve and the displacement ratio should approach unity. The BGE should also have an electrophoretic mobility close to that of the analytes to maximize sensitivity and minimize dispersion effects that can arise from the electrophoretic mobility differences between the analyte and the BGE.[50]

CZE with indirect UV detection at 254 nm was first used for the detection of a series of alkylphosphonic acids.[51] Four BGEs were investigated, benzoic acid, phthalic acid, sorbic acid, and phenylphosphonic acid. Phenylphosphonic acid, which has a mobility close to that of the analyzed compounds, was shown to be the most suitable BGE. The influence of several parameters, such as the concentration of the BGE and the borate buffer on both sensitivity and efficiency, was investigated. An increase in the borate concentration produced an improvement of the signal-to-noise ratio (S/N) while the sensitivity decreased with increasing concentration of the phenylphosphonic acid. At pH 6, 200 mM sodium borate buffer containing 10 mM phenylphosphonic acid was selected and the separation of MPA, EPA, PPA, and BPA was demonstrated. The reproducibility of the μ_e for

Table 1 CWC-related organophosphorus compounds analyzed by CE

Structure	R_1	R_2	Name	CAS No.	Refs.
Alkylphosphonic acids [CH_3, CH_3CH_2, CH_3CH_2, $(CH_3)_2CH$]:					
	H	CH_3	MPA	363-06-7	51, 52, 54–57, 60, 76, 79
	H	CH_3CH_2	EPA	1782-26-4	51, 52, 55, 60, 76
	H	$CH_3CH_2CH_2$	nPPA	4672-38-2	51, 55, 56, 60
	H	$(CH_3)_2CH$	iPPA	4721-37-3	77
O-Alkyl (≤C_{10} including cycloalkyl) alkylphosphonates:					
	CH_3	CH_3	MMPA	1066-53-1	53, 55
	CH_3CH_2	CH_3	EMPA	1832-53-7	52–57, 60, 76
	$(CH_3)_2CH$	CH_3	IMPA	1832-54-8	52–57, 60, 76, 79
	Cyclohexyl	CH_3	CHMPA	1932-60-1	52, 53, 56, 60, 76
	$(CH_3)_2CHCH_2$	CH_3	iBMPA	1604-38-2	76
	Pinacolyl	CH_3	PMPA	616-52-4	52–58, 76, 79
	$(CH_3)_2CH(CH_3)CH$	CH_3	DMPA	151299-67-1	52, 53
	Cyclopentyl	CH_3	CPMPA	73207-99-5	56
	2-Ethylhexyl	CH_3	EHMPA	13688-82-9	76
	CH_3CH_2	CH_3CH_2	EEPA	7305-61-5	52, 53
	$(CH_3)_2CH$	CH_3CH_2	iPEPA	170135-50-9	53, 76
	$(CH_3)_2CH(CH_3)CH$	CH_3CH_2	DEPA	195158-11-3	53
	Cyclohexyl	CH_3CH_2	CEPA	170424-87-0	52, 53
Methylphosphonothioic acids:					
	CH_3CH_2	H	EMPSA	18005-40-8	56, 57, 60, 61
	$(CH_3)_2CHCH_2$	H	IBMPSA	20626-99-7	60, 61
	H	$(iPr)_2CH_2CH_2N$	EA 2192	73207-98-4	60

MPA was 1.65% (relative standard deviation) and the LOD was <10 µM (0.21 pmol injected).

Sorbic acid has also been shown to be a suitable BGE for the analysis of the alkylphosphonic acids and their monoester derivatives.[52] A run buffer consisting of 5 mM sorbic acid and 0.1 M decamethonium bromide, adjusted to pH 6, was used to separate a mixture of eight alkyl alkylphosphonates. The use of an EOF modifier, decamethonium bromide, reduced the EOF at pH 6 and gave complete separation of the alkylphosphonic acids and their monoester derivatives (e.g. MPA and IMPA) in <15 min (Figure 2).

To reduce run times while maintaining high peak efficiencies, the capillary can be dynamically coated with a cationic polymer to reverse the EOF. In the procedure the column is flushed with a polymer solution prior to each analysis. This approach has been demonstrated with a 0.2% aqueous solution of polybren.[53] Sorbic acid was used as the BGE and hydroxylamine was added to improve the buffer capacity of the run buffer. Using an electrolyte composed of 5 mM sorbate anion (pH 6) and 1.6 mM hydroxylamine allowed the separation of alkylphosphonic acid monoesters in <6 min. Chromate

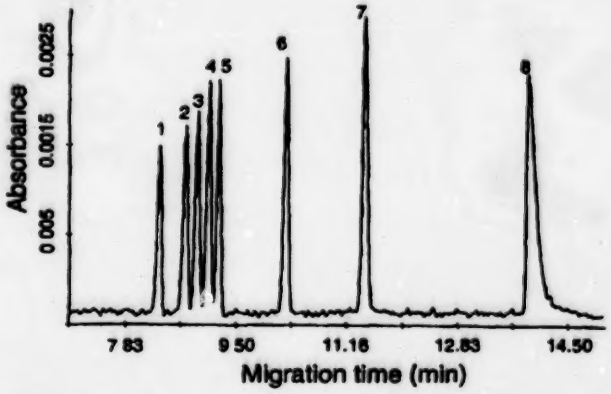


Figure 2 CZE of a standard mixture (solute concentration 20 mg L^{-1}) of eight alkylphosphonic acid anions: CEPA (1), PMPA (2), CMPA (3), DMPA (4), EEPA (5), IMPA (6), EMPA (7), and MPA (8). CZE analysis conditions: 67 cm L_t (60 cm L_d) × 50 µm i.d.; V = 30 kV; 5 mM sorbic acid, 0.1 mM decamethonium bromide, pH 6; indirect UV detection at 254 nm; 5 s hydrodynamic injection. (Reprinted from J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambute, *J. Chromatogr. A*, 741, 279–285 (1996), Copyright © 1996, with permission from Elsevier Science.)

has also been used as a BGE in the presence of a proprietary EOF modifier for the separation of MPA, EMPA, IMPA, and PMPA.[54]

4.2.2 Direct Ultraviolet Detection

The direct UV detection of alkylphosphonic acids through the formation of sodium borate complexes has been reported.[55] The buffer system consisted of 10 mM sodium tetraborate with complexation occurring in solution at pH 7 and higher. The reported detection limits were of the order of nanograms. The UV, Infrared (IR), NMR, and mass spectra of various borate–phosphonic acid esters were also reported.

4.2.3 Flame Photometric Detection

The separation performance and sensitivity of a CE system coupled on-line with a flame photometric detector (FPD), originally developed for GC, have been reported for the detection of alkylphosphonic acids.[56] The liquid junction used to decouple the electric field from the FPD showed a negligible influence on the performance of the system as compared with online UV detection. Use of an on-column sample stacking pre-concentration technique allowed for injection of 900 nL. With the large injection, the detection limits for the alkylphosphonic acids in water were 0.1–0.5 $\mu g\,mL^{-1}$.

4.2.4 Mass Spectrometric Detection

MS is the only technique which to date has been coupled with CE to provide the structural information required for unambiguous identification of CW degradation products. Capillary zone electrophoresis/ionspray mass spectrometry (CZE/ISMS) combines the high separation efficiency of CE and the high sensitivity of MS for the analysis of these polar degradation products. CZE/ISMS, in the negative-ion mode, was applied to the identification of five organophosphonic acids which are the primary hydrolysis products of nerve agents.[57] The spectra of EMPA, IMPA, and PMPA exhibited very abundant $[M-H]^-$ ions of *m/z* 123, 137, and 179, respectively. The fragmentation was minimal and common fragmentation ions at *m/z* 95 and 79 were observed due to the loss of the alkyl and alkoxy groups, respectively. Sensitivity in the range 10–30 pg (5 $ng\,\mu L^{-1}$) was achieved using selected ion monitoring (SIM).

4.3 Sulfur-containing Compounds

4.3.1 Micellar Electrokinetic Chromatography

MEKC is a combination of electrophoresis and chromatography.[58,59] The main advantage of MEKC is that it can be used for the separation of both neutral and charged solutes. This is done by adding a surfactant such as sodium dodecylsulfate (SDS) at concentrations above the critical micelle concentration to the running buffer to form micelles. Neutral species partition in and out of the micelle. Since the micelles are charged, this partitioning imparts a mobility to the neutral species. The more hydrophobic the analyte, the more it is retained within the micelle and the more it is affected by the charge of the micelle. Since MEKC can be used for both charged and uncharged species, its applications include analyses of amino acids, nucleotides, vitamins, pharmaceuticals, aromatic hydrocarbons, and explosive constituents.

The sulfur-containing, neutral degradation products of mustard and related vesicants can be analyzed by direct UV detection using MEKC. MEKC has been used for the analysis of thiodiglycol (TDG), 2,2'-sulfinyldiethanol (TDGO), 1,4-dithiane, 1,4-thioxane, *O*-isobutyl methylphosphonothioic acid (IBMPSA) and *O*-ethyl methylphosphonothioic acid (EMPSA). These were separated in under 10 min with a run buffer of 10 mM borate and 100 mM SDS at pH 9 (Figure 3).[60,61] Detection limits range from 1 to 10 $\mu g\,mL^{-1}$. Sulfur-containing compounds related to the decomposition of sulfur mustard and other materials analyzed by CE with direct UV detection are listed in Table 2.

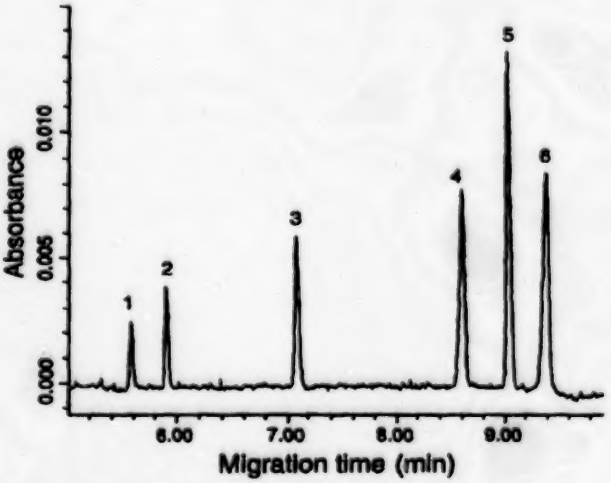


Figure 3 MEKC of six sulfur-containing compounds related to the degradation of mustard and *O*-alkyl alkylphosphonothiolates (solute concentration 50 $mg\,L^{-1}$): TDGO (1), TDG (2), thioxane (3), iBMPSA (4), dithiane (5), and EMPSA (6). MEKC analysis conditions: 57 cm L_t (50 cm L_d) × 75 μm i.d.; $V = 25$ kV; 10 mM borate, 100 mM SDS, pH 9; direct UV detection at 200 nm; 3 s hydrodynamic injection. (Reprinted from J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambute, *J. Chromatogr. A*, 741, 279–285 (1996), Copyright 1996, with permission from Elsevier Science.)

Table 2 CWC-related degradation products of sulfur mustard, lewisite and other compounds analyzed by CE

Structure	Name	CAS No.	Refs.
Sulfur mustard-related compounds:			
$HOCH_2CH_2SCH_2CH_2OH$	TDG	111-48-8	60, 61
$HOCH_2CH_2S(O)CH_2CH_2OH$	TDGO	3085-45-8	60, 61
$HOCH_2CH_2S(O)_2CH_2CH_2OH$	$TDGO_2$	2580-77-0	60
	1,4-Thioxane	15980-15-1	60, 61
	1,4-Dithiane	505-29-3	60, 61
	1,4-Dithiane-1-oxide	19087-70-8	60
Lewisite-related compounds:			
ClCHCHAs(O)	CVAOA	3088-37-7	60
Schedule 2B precursors			
$(CH_3CH_2)_2NCH_2CH_2OH$	DEAE	100-37-8	60
$[(CH_3)_2CH]_2NCH_2CH_2OH$	DIPAE	96-80-0	60
$[(CH_3)_2CH]_2NCH_2CH_2SH$	DIPAESA	1439-97-5	60

4.4 Toxins

4.4.1 Saxitoxin

STX, Schedule 1.A.(7), is a potent neurotoxin which can cause paralytic shellfish poisoning (PSP). STX is produced by certain strains of dinoflagellates, which leads to the contamination of commercial shellfish and causes severe outbreaks of seafood poisoning. The public health problems caused by these outbreaks have led to significant interest in the development of analytical methods for the determination of STX in environmental and biological samples. STX is also one of several closely related compounds and separation and so unambiguous identification of the target compound is a significant analytical requirement for its analysis.

STX is one of the only CWC-related compounds which has been analyzed by CZE with LIF detection. Peptides and proteins containing lysine residues or natural products which contain primary amino groups (NH_2) can be derivatized with a number of fluorescent labeling reagents.[62] LIF is an extremely sensitive detection method and can provide CLODs in the 10^{-11}–10^{-13} M range.[63] STX has been labeled with fluorescamine, *o*-phthaldialdehyde (OPA) and dansyl chloride and detection limits as low as 0.1 amol were reported for the OPA derivative of STX.[64] However, the precolumn labeling methods required micromolar concentrations of analyte, limiting the utility in trace analysis. Labeling, separation, and analysis of STX were best accomplished using the fluorescamine derivative, which produces ionic derivatives that can be separated from other fluorescently labeled marine toxins such as tetrodotoxin and microcystin.

CE analysis with direct UV absorbance detection at 200 nm has been described for the separation and detection of underivatized toxins, including STX, associated with PSP.[65] Confirmation of the electrophoretic peaks was made by capillary electrophoresis/electrospray ionization mass spectrometry (CE/ESIMS). STX and neosaxitoxin (NEO) were separated using 20 mM sodium citrate buffer (pH 2.1) with a mass detection limit of 15 pg (5 μM) for STX. This method was able to detect STX and NEO algal and mollusc extracts. Another reported UV method uses 208 nm and a 0.06 M sodium borate buffer for the analysis of STX in molluscs.[66]

The application of on-column sample preconcentration CITP and a discontinuous buffer system prior to CE separation has been investigated for the analysis of PSP toxins. Using a linear polyacrylamide-coated capillary to eliminate EOF during the CITP step, 35 mM morpholine (pH 5) as the leading electrolyte and 10 mM formic acid as the terminating electrolyte, the improvement of the CLOD is at least two orders of magnitude over that obtainable using the conventional CZE format.[67] Analyses performed using SIM ESIMS provided a CLOD of 16 nM for STX. This preconcentration technique was compatible with ESIMS and permitted the analysis of biological extracts containing PSP toxin levels as low as

100 nM. In situations where higher levels of selectivity are required for unambiguous identification of individual PSP toxins, CITP or CZE with MS/MS can be used. Further improvements in sample focusing prior to CE separation can be obtained using high-ionic-strength buffers and on-column sample stacking.[68]

4.4.2 Ricin

CE has found particular application in the analysis of peptides[69,70] and proteins.[71,72] The resolution and ease of operation combined with the various CE modes such as CGE have led to a tremendous number of applications for CE analysis of amino acids, peptides, and proteins. Proteins are readily analyzed by direct UV detection at 200 nm. CE analysis of proteins can present additional challenges such as sample microheterogeneity due to variations in carbohydrate structure, impurities from the isolation or preparation of the protein sample, or surface adhesion to the capillary walls. This can result in multiple peaks or significant peak broadening, peak tailing, and even irreversible loss of protein.

Ricin is a heterodimeric glycoprotein (MW = 66 000) produced by the castor bean plant *Ricinus communis* with the two protein subunits linked by a single disulfide bond. Conditions for the analysis of ricin with CE, using uncoated and coated columns, and the influence of buffer systems (run buffer composition, pH, ionic strength, and additives) have been investigated. Uncoated columns used with either zwitterionic salts or putrescine gave the best results.[73,74] Multiple peaks were resolved with these conditions. Analysis using a bare fused-silica column with a 0.03 M sodium borate run buffer gave a single, broad, tailing peak which could be due to protein–column interactions, additional components, or both. The use of 0.03 M putrescine (1,4-diaminobutane) in 0.02 M K_2SO_4 buffer (pH 7) gave better resolution and peak shape and multiple peaks were resolved, indicating possible impurities or microheterogeneity in the ricin sample.

5 APPLICATIONS

5.1 Environmental Analysis

The development of CE analytical methods for CW-related compounds has been directed primarily towards the analysis of these compounds in environmental matrices such as soil and water. These methods have been used in the analysis of samples distributed as part of interlaboratory comparisons exercises, the so-called Finnish Round Robin Exercises[38] and more recently the Organization for the Prohibition of Chemical Weapons (OPCW) Laboratory Proficiency Testing.[75]

The use of CE with indirect UV detection in environmental samples leads to matrix effects from inorganic ions that comigrate. Surface or groundwater samples can contain carbonate levels that interfere with alkylphosphonate analysis. Ion-exchange cartridges such as the Milli-Trap H^+ (Waters) can be used to reduce carbonate levels and Ag^+ resins can be used to remove chloride ions.[59] Using decamethonium bromide to reduce the EOF, CEPA- and EPA-spiked soil samples, extracted with water, could be analyzed without significant interferences.[57] However, differences in the migration times were noted owing to matrix effects in the soil extracts and required the use of a standard to achieve quantitative results. Environmental water samples, spiked with standard mustard degradation products, have been analyzed by MEKC with direct UV detection with no apparent interferences or requirement for sample pretreatment.[66]

As part of an Interlaboratory Proficiency Test, CE with FPD was used to screen water samples and aqueous extracts of soil, rubber, and paint for spiked alkylphosphonic acids.[61] The water sample had been spiked with PPA at a level of 8 $\mu g\,mL^{-1}$ and one soil sample was spiked with CEPA and EPA at 15 and 8 $\mu g\,g^{-1}$, respectively. Although the recovery efficiency was only approximately 20%, CEPA and EPA could still be detected using longer injection volumes and higher on-column concentrations. Subsequent NMR and *tert*-butyldimethylsilyl derivatization and GC/MS analysis confirmed the identity of the phosphorus-containing compounds in the extract.

Reversal of EOF was used in the analysis of samples in the second and third OPCW Laboratory Proficiency Tests.[76] The cationic surfactants didodecyldimethylammonium hydroxide and cetyltrimethylammonium hydroxide were used to reverse the EOF and remove interferences from common anions and give complete and rapid separation of alkylphosphonic acids. EPA and PEPA were detected in water samples as part of the second OPCW test and PPA and EHMPA were detected in the water samples as part of the third OPCW test. Identification of spiked compounds was first made on the basis of mobility data and confirmed by MS. The method was applied to aqueous leachates of soil, wipes of surfaces, and vegetation sampled from a field site known to have been exposed to nerve agents and subsequently remediated. MPA, IMPA, and PMPA were detected in these samples, indicating that the method can be useful for environmental monitoring.

5.2 Quality Assurance

Under the terms of the CWC, the Director General of the OPCW shall establish stringent regimes governing the collection, handling, transport, and analysis of

Table 3 Representative CE methods for the analysis of CW degradation products

Class	Mode	Buffer	pH	Detection: λ (nm)	E ($V\,cm^{-1}$)	Ref.
Alkylphosphonates	CZE	200 mM borate 10 mM phenylphosphonic acid[a]	6	Indirect UV (254)	465	51
Alkylphosphonates	CZE	5 mM sorbic acid[a] 0.1 mM decamethonium bromide	6	Indirect UV (254)	448	52
Alkylphosphonates	CZE	200 mM boric acid 10 mM phenylphosphonic acid[a] 0.03 wt% Triton X 0.35% didodecyldimethylammonium (DDAOH)	4	Indirect UV (210)	536	79
Alkylphosphonates	CZE	20 mM ammonium acetate	9	ISMS	375	57
Methylphosphonothioates	CZE	250 mM borate	7	Direct UV (200)	470	60
S-Mustard-related compounds	MEKC	10 mM borate 100 mM SDS	9	Direct UV (200)	440	61

[a] BGE.

samples. The Director General shall also certify laboratories designated for the analysis of samples, oversee standardization of procedures, and monitor quality control standards at certified laboratories. To be certified as a "Designated Laboratory" by the OPCW under the terms of the CWC, a laboratory must have established an internationally recognized quality assurance system, obtained accreditation by an internationally recognized accreditation body for which they are seeking accreditation, and regularly participate and perform successfully interlaboratory proficiency tests.[77] A requirement for accreditation of testing laboratories under such standards as the "General Criteria for the Operation of Testing Laboratories", European Standard 45 001: European Standards Institution, Brussels EN 45 001, is that the laboratory follow written standard operating procedures and implement a quality assurance/quality control program.

In addition to demonstrating the role of analytical methods in verification of the CWC, the round-robin methods have led to the publication of recommended operating procedures which can be used for the analysis of the target compounds. The published method of Pianetti et al.[51] has been put forward as a recommended operating procedure for the analysis of the alkylphosphonates.[78] Other representative methods for the analysis of various CWC-related compounds which could form the basis of a standard or recommended operating procedure in a designated laboratory are summarized in Table 3.

A validated quantitative analysis for CW degradation products has been developed.[79] The specificity, linearity, precision, and accuracy were examined for the analysis of IMPA and PMPA using two buffer systems and detection methods. The method gave inter- and intra-day reproducibility of 1–3% (relative standard deviation). The method requires little sample preparation except for dilution with deionized water and is not prone to interference from carbonate or fluoride ions.

6 CONCLUSION

The ability of CE to analyze anionic, cationic, zwitterionic, and neutral CWC-related compounds, whether they be CW degradation products or scheduled starting materials, without the requirement for elaborate sample processing or labeling procedures is an important demonstration of the utility of CE for the analysis of CWC-related compounds. CE can be an important screening method for rapid sample processing and further analysis for unambiguous identification. The relative ease of analysis should also increase its utility in field and on-site analysis where minimizing the logistic burden is attractive. The increased use of MS coupled techniques and the advent of CE coupled with NMR[80] will also serve to establish CE as an important method for the unambiguous identification of CWC compounds.

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ABBREVIATIONS AND ACRONYMS

BGE	Background Electrolyte
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CE/ESIMS	Capillary Electrophoresis/Electrospray Ionization Mass Spectrometry
CGE	Capillary Gel Electrophoresis
CIE	Capillary Ion Electrophoresis
CIEF	Capillary Isoelectric Focusing
CITP	Capillary Isotachophoresis
CLOD	Concentration Limit of Detection
CW	Chemical Warfare
CWC	Chemical Weapons Convention
CZE	Capillary Zone Electrophoresis
CZE/ISMS	Capillary Zone Electrophoresis/ Ionspray Mass Spectrometry
EOF	Electroosmotic Flow
ES	Electrospray
ESIMS	Electrospray Ionization Mass Spectrometry
FPD	Flame Photometric Detector
GA	Tabun
GB	Sarin
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
GD	Soman
GF	Cyclohexyl Sarin
HD	Mustard Gas
HN-1	Ethyl Nitrogen Mustard
HPLC	High-performance Liquid Chromatography
IC	Ion Chromatography
IR	Infrared
IS	Ionspray
LIF	Laser-induced Fluorescence
LOD	Limit of Detection
MEKC	Micellar Electrokinetic Chromatography
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NEO	Neosaxitoxin
NMR	Nuclear Magnetic Resonance
OPA	*o*-Phthaldialdehyde
OPCW	Organization for the Prohibition of Chemical Weapons
PSP	Paralytic Shellfish Poisoning
SDS	Sodium Dodecylsulfate
SIM	Selected Ion Monitoring
S/N	Signal-to-noise Ratio
STX	Saxitoxin
UV	Ultraviolet
VX	*O*-Ethyl *S*-2-diisopropylaminoethyl-methyl Phosphonothiolate

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